

The Emergence of *bla*_{-CTX-M} and *bla*_{-TEM} in ESBL Producing *Klebsiella pneumoniae* in Aquaculture in Southeast Asia: A Systematic Review

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ABSTRACT

ESBL *Klebsiella pneumoniae* contributes widely to the alarming increase observed globally in antibiotic resistance within the past few years. The aquaculture sector is known as a fast-emerging business today. It is one of the significant concerning sectors with the emerging ESBL-producing *K. pneumoniae* due to its complex treatment and easy-transfer genes. This mini-review aims to determine the emergence of *bla*_{-CTX-M} and *bla*_{-TEM} genes in ESBL-producing *K. pneumoniae* found in aquaculture within Southeast Asia. All qualified journals considered have been conscientiously examined and passed the mini-review criteria; 8 eligible studies from Southeast Asia from 2016 to 2021 were utilized. Among the eight journals, five out of the eight studies have displayed the detection of the *bla*_{-CTX-M} gene found within freshwater, fish markets, and integrated farming systems in Thailand and India, with a prevalence of 44.38% and 33.73%, respectively. The remaining three studies display *bla*_{-TEM} genes found in freshwater, retail markets, and freshwater aquaculture in Hongkong and the Philippines, with a prevalence of 19.53% and 2.37%. Within Southeast Asia, 37.50% account for the *bla*_{-TEM} gene, while 62.50% account for *bla*_{-CTX-M}, making it the most pervasive gene found within Southeast Asia. Infections caused by ESBL-producing *K. pneumoniae* can be resistant to various antibiotics, complicating the treatment process. Observing proper hygiene and taking extra precautions can significantly reduce ESBL-producing *K. pneumoniae* emergence.

Keywords: Aquaculture, *bla*_{-CTX-M}, *bla*_{-TEM}, Extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae*.

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INTRODUCTION

Beta-lactamase is an enzyme produced by a specific type of bacteria that opens the β -lactam ring and inactivates the β -lactam antibiotics. Extended-Spectrum-Beta-Lactamase-producing *Klebsiella pneumoniae* produces a substantial influence on antimicrobial resistance bacteria because of their capacity to transfer genes to other bacteria.^[1] *K. pneumoniae* is also known to harbor

*bla*_{-CTX-M} and *bla*_{-TEM}. These are some of the various ESBL genes that can be detected in environmental waters in which *K. pneumoniae* is one of the most common ESBL-producing isolates found in Asian lakes and rivers.^[2] Because of that, β -lactam antibiotics are widely used for intensive animal production and growth promotion, especially in aquaculture.^[3]

Aquaculture is a fast-expanding food business, with the bulk of production occurring in poor and middle-income nations.^[4] However, the misuse of antibiotics in animals has resulted in the widespread dissemination of ESBL-producing *K. pneumoniae*.^[5] According to the World Health Organization (WHO), there is an alarming widespread of ESBL bacteria such as *E. coli* and *K. pneumoniae*. WHO integrated surveillance on antimicrobial resistance to target the monitoring

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of ESBL producing *E. coli* and *K. pneumoniae* in both humans and animals, which can lessen the spread of resistance mechanisms.

ESBL causes clinical antimicrobial treatment to fail, and it has become a significant public health problem across the world.^[6] This systematic review aims to demonstrate the prevalence of bla_{-CTX-M} and bla_{-TEM} in ESBL producing *Klebsiella pneumoniae* in Aquaculture in Southeast Asia and determine their transmission from aquaculture to humans.

METHODS

Literature Search

A systematic review of English-language publications on ESBL-producing *Klebsiella pneumoniae* in Southeast Asian aquaculture was created using four online databases: Google Scholar, EBSCOHost, PubMed, ScienceDirect, and Research Gate. The articles were chosen based on the relevance of the title, abstract, and the full version of the article. The publication years of the articles range from 2016 to 2022. The keywords were used in combinations and contained the Boolean operators “OR” and “AND.” The keywords used were ‘Southeast Asia OR SEA,’ ‘Extended-spectrum beta-lactamase OR ESBL,’ ‘*Klebsiella pneumoniae* OR *K. pneumoniae*,’ ‘Aquaculture,’ ‘bla_{-CTX-M}’ and ‘bla_{-TEM}’ The references used in the study were processed using the Mendeley Reference Manager.

Eligibility of Criteria

Eligible papers included in this review were studies describing the presence and prevalence of genes bla_{-CTX-M} and bla_{-TEM} in ESBL-producing *Klebsiella pneumoniae* recovered from aquaculture or farmed aquatic organisms in Southeast Asia. Included studies also describe in detail the transmission pattern of these ESBL genes from aquaculture to humans. The study design used for the qualified epidemiologic studies is mostly cross-sectional and surveillance studies to observe, identify, and analyze the presence and prevalence of ESBL-producing *K. pneumoniae* genes with high antibiotic resistance. ESBL-producing bacteria and other genes, antimicrobial profiling, biochemical mechanism, cross-contamination, and detection methodologies were excluded from the selection of reference studies. To present up-to-date data, only studies published from 2016 onwards were eligible.

Data Selection and Extraction

All published journals and research articles gathered by the researchers collectively discussed the prevalence

of bla_{-CTX-M} and bla_{-TEM} genes in ESBL-producing *Klebsiella pneumoniae* present in aquaculture within countries in Southeast Asia. All essential data that leads to the demonstration of bla_{-CTX-M} and bla_{-TEM} genes in aquaculture have been extracted and evaluated independently following the eligibility criteria. The data set of this review was summarized using MS Excel, which includes the title of the article/paper, the name of the author(s), and the year of publication.

RESULTS

Characteristics of Included Studies

Figure 1 illustrates that 123 publications were screened through several databases using the keywords in this study. One article was removed as duplicate titles, and 54 journals were chosen after further screening to ensure that all selected publications were related to the topic and title of the review. After thorough checking, the 52 publications that were considered eligible were further reduced based on the inclusion and exclusion criteria. Out of the 52 eligible publications, only eight have met the criteria and were used in the review. On the other hand, the remaining 44 publications are not qualified since most of these studies have focused more on other ESBL-producing bacteria aside from *K. pneumoniae*, some are not limited to Southeast

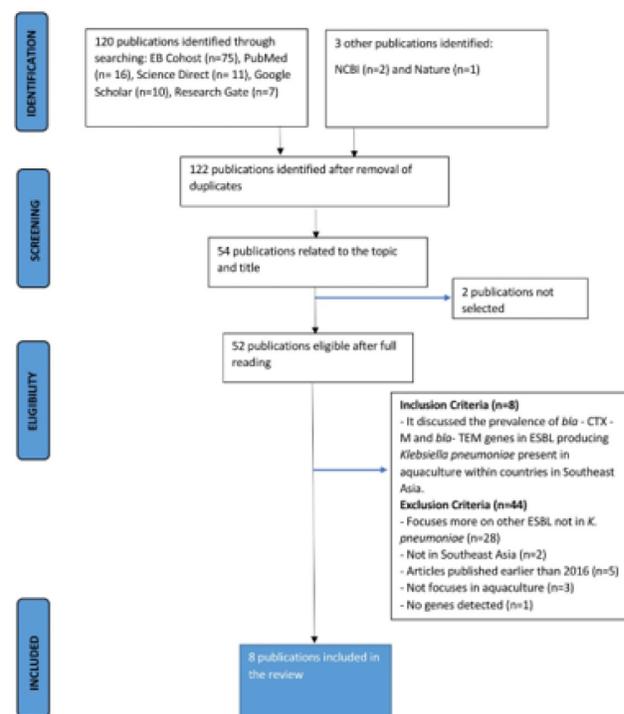


Figure 1: Journal selection process.

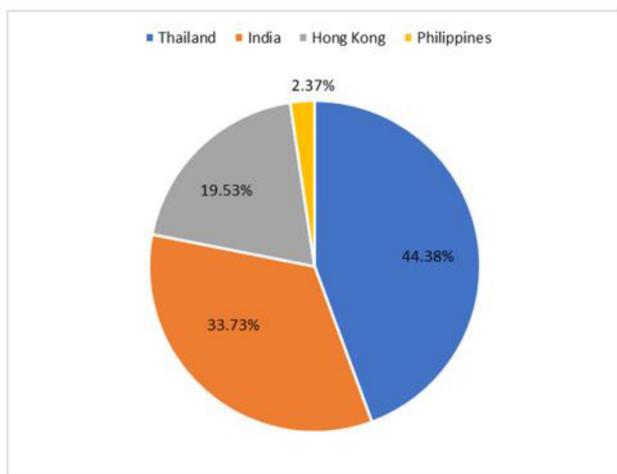


Figure 2: Prevalence of ESBL producing *Klebsiella pneumoniae* in Aquaculture in Southeast Asia.

Asia and aquaculture only, and lastly, *bla*_{CTX-M} and *bla*_{TEM} genes were not detected.

ESBL producing *Klebsiella Pneumoniae* in Aquaculture in Southeast Asia

By the findings of the eight journals included, it was concluded that India, Thailand, the Philippines, and Hong Kong were deemed positive in containing ESBL producing *K. pneumoniae*, which were detected in their aquaculture. Based on the data shown in Figure 2, Thailand has the highest proportion of ESBL producing *K. pneumoniae* among the other Southeast Asian countries, while the Philippines has the lowest. Among the 169 isolates confirmed positive for the ESBL genes, 44.38% were from Thailand, 33.73% from India, 19.53% from Hong Kong, and 2.37% from the Philippines.

Detected Genes in ESBL Producing *K. pneumoniae*

Based on the results of included journals shown in Figure 3, 62.50% of *bla*_{CTX-M} genes are detected in ESBL producing *K. pneumoniae*, which is the most prevalent gene in Southeast Asia. In comparison, 37.50% of *bla*_{TEM} genes are detected in ESBL producing *K. pneumoniae*.

DISCUSSION

The aquaculture industry is widely regarded as the world's fastest-growing food source. Bacterial infections are one of the most common problems in the sector. They induce diseases in various species in fish farms which can impose health risks on humans due to increased transmission and consumption of products.^[7] To resolve such issues, antibiotics are used as a treatment for bacterial infections as well as a growth stimulant

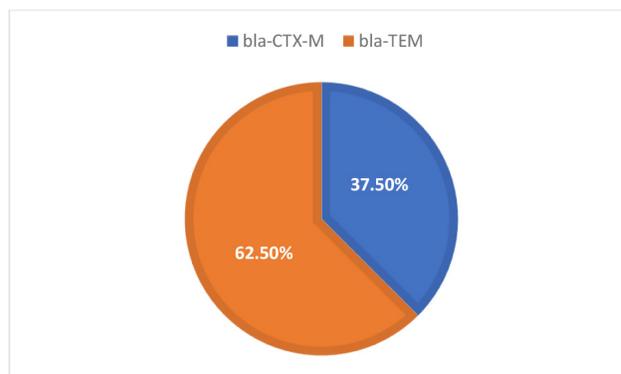


Figure 3: Percentage of *bla*_{CTX-M} and *bla*_{TEM} genes.

due to the increased demand for fish products, and prophylactics are also used to prevent infections.^[4]

Another challenge the sector encounters is the release of antibiotics into the aquatic environment through hospital discharges, domestic sewage, and antibiotic residuals from other fish farms or aquatic environments. Beta-lactam antibiotics are among the most frequently used in the aquaculture industry and are considered a global health risk due to their extensive use. Bacteria that are resistant to these antibiotics may carry different β -lactamase genes that may encode for the enzyme called extended-spectrum β -lactamase.^[2] ESBLs are generally resistant to third-generation cephalosporins and are inhibited by clavulanic acid, which is a β -lactamase inhibitor. Based on earlier studies, the most prevalent gene commonly identified in ESBL-producing *K. pneumoniae* from aquatic animals is *bla*_{CTX-M}.^[7] *K. pneumoniae* is the most prevalent bacteria identified from various fish farms. It is known to be an important food pathogen since it is a major cause of multiple diseases in humans, including pneumonia, septicemia, and urinary tract infections. As a result, an increase in the usage of antibiotics in aquaculture may lead to the emergence of antibiotic or antimicrobial resistance and eventually result in multiple drug resistance.^[7] If an isolate has resistance to at least one agent from more than three antimicrobial groups, it is classified as Multiple Drug Resistance (MDR).^[8]

The present study discovered that *K. pneumoniae* isolates have a significant resistance rate to therapeutically important antimicrobials, including penicillin, third-generation cephalosporins (cefotaxime, cefazolin, ceftazidime, and ceftriaxone), and the monobactams drug aztreonam.^[7] According to Arumugham *et al.*, cephalosporins, like beta-lactam antibiotics, prevent bacterial cell wall formation. The β -lactam ring structure of 3rd generation cephalosporins mimics the "D-Ala-D-Ala" moiety of penicillin-binding proteins

(PBPs) natural substrate. Cephalosporin antibiotics bind structurally to the active site of bacterial cell walls of PBPs, preventing their enzyme activities and inducing defective peptidoglycan production, causing bacterial cell death by osmotic lysis.^[9]

Based on previous studies, two genes that are commonly identified among different species in aquacultures are bla_{-CTX-M} and bla_{-TEM}. However, it is revealed that bla_{-CTX-M} genes have a significantly higher prevalence than bla_{-TEM} genes. Based on the study of Bonomo, one of the most used antibiotics is cefotaxime, a β -lactam antibiotic that works by blocking the enzymes involved in cell wall synthesis, such as penicillin-binding proteins (PBPs), to kill bacteria. However, the bla_{-CTX-M} gene of *K. pneumoniae* is a Class A β -lactamase that exhibits resistance to cefotaxime because β -lactamases hydrolyze the antibiotic and inactivate the antibiotic molecules and thus, making it difficult to treat the infected species with the specific gene causing its high prevalence.^[10]

According to the study by Sivaraman *et al.*, there is a 100% prevalence of the bla_{-CTX-M}-15 gene in the aquaculture farms in Kerala, India.^[4] This specific ESBL-producing *K. pneumoniae* gene was isolated from *Litopenaeus vannamei* and *Penaeus monodon*. Similarly, there is an 86.7% prevalence of the said genes that were isolated from *Oreochromis niloticus* found in fish farms and 53.85% from the same gene and species found in well-type farms, floating farms, and nursery farms in Thailand and those that are found in Mula River, India had 38%.^[7,11-12] Species such as *Hypobalmichthys molitrix*, *Labeo rohita*, *Pygocentrus nattereri*, and *Catla* found in rivers and ponds in Assam, India, exhibited a 100% prevalence of bla_{-CTX-M} gene.^[8]

On the other hand, the genes: bla_{-TEM}-1, bla_{-TEM}-116, and bla_{-TEM}-206 showed a 36.8% prevalence in which it is identified from species found in freshwater aquaculture such as lakes and rivers in the Philippines.^[2] In addition, the study by Singh *et al.* stated that species such as fishes, shrimps, clams, and squids found in retail markets in India showed a 65.21% prevalence of the bla_{-TEM} gene.^[13] Furthermore, in Hong Kong's wet markets, snakeheads, which are freshwater fish, have a prevalence of 19.3% of the same gene.^[1]

CONCLUSION AND RECOMMENDATION

Based on a limited number of studies, the pooled prevalence of ESBL producing *Klebsiella pneumoniae* isolated genes detected from various freshwater fishes obtained from aquaculture resources in Southeast Asia is relatively high compared to other gene groups. In conclusion, India is the country in Southeast Asia that

is more prevalent in ESBL-producing *K. pneumoniae* in aquaculture due to the considerable amount of detected ESBL-producing *K. pneumoniae*. In addition to that, according to the mentioned journals, bla_{-CTX-M} is the most prevalent gene detected in ESBL producing *K. pneumoniae*. Still, bla_{-TEM} is also evident in ESBL producing *K. pneumoniae*. These genes are responsible for the antimicrobial resistance of the bacteria to several types of antibiotics present on the market. The increasing proportion of these ESBL resistance genes is becoming more of a risk and threat to the aquaculture industry as more seafood, which humans commonly consume, is primarily affected. Further investigation and analysis on the prevalence of these ESBL resistant genes on aquaculture species are required as there is insufficient data regarding the growing burden of ESBL bla_{-CTX-M}. Future researchers may study other types of specific genes related to the antimicrobial resistance of *K. pneumoniae*. Several factors have contributed to the widespread contamination, development, and distribution of antimicrobial resistance, including inadequate sanitation and hygiene practices in the marketplaces, misuse of antibiotics in animals, cross-contamination, and biochemical mechanisms, which may contribute to the widespread dissemination of ESBL producing *K. pneumoniae*. They are emerging pathogens and a burden that must be studied with adequate surveillance as they can be significant for creating preventive measures and improving the aquaculture industry.

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Authors' Contribution

In completing this study, all researchers equally contributed and collected journals for utilization in this study. In writing the introduction, it is done with the assistance of researchers A.A. and D. A. Researchers D.R., GN., N.S., P.G., S.G., and S.M. took the lead in explaining the methodology. All researchers contributed to discussing the results and discussion of the study with the direction and complete guidance of A.A. and

T.P. alongside proofreading. With all of these efforts, the final manuscript of the mini-review is completed.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

SUMMARY

ESBL *K. pneumoniae* is a major contributor to the upsurge in antibiotic resistance seen globally in recent years and it greatly influences the fast-emerging aquaculture sector due to the misuse of antibiotics that resulted in large-scale distribution of ESBL producing *K. pneumoniae*. As a result, it harbors different genes that gives a big influence on antimicrobial resistance. In making this review, journals are done through selection criteria to ensure their cogency. The journals are collected from accredited references. By the findings of the study, it is revealed that Thailand has the highest proportion of ESBL producing *K. pneumoniae* while the lowest in the Philippines. *K. pneumoniae* harbors different genes such as bla_{-CTX-M} and bla_{-TEM}. The results show that bla_{-CTX-M} is the most prevalent gene because this gene is a Class A β -lactamase making it more resistant.

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